Marine Pollution Bulletin 134 (2018) 14–26

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Interactive effect of temperature, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa*

L.G. Egea*, R. Jiménez-Ramos, J.J. Vergara, I. Hernández, F.G. Brun

Department of Biology, Faculty of Marine and Environmental Sciences, University of Cadiz, 11510, Puerto Real, Cadiz, Spain

ARTICLE INFO

Keywords:
Acidification
Cymodocea nodosa
Eutrophication
Climate change
Carbon dioxide
Warming

ABSTRACT

Global (e.g. climate change) and local factors (e.g. nutrient enrichment) act together in nature strongly hammering coastal ecosystems, where seagrasses play a critical ecological role. This experiment explores the combined effects of warming, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa* under a full factorial mesocosm design. Warming increased plant production but at the expense of reducing carbon reserves. Meanwhile, acidification had not effects on plant production but increased slightly carbon reserves, while a slight stimulation of net production and a slight decrease on carbon reserves under ammonium supply were recorded. When all the factors were combined together improved the production and carbon reserves of *Cymodocea nodosa*, indicating that acidification improved ammonium assimilation and buffered the enhanced respiration promoted by temperature. Therefore, it could indicate that this temperate species may benefit under the simulated future scenarios, but indirect effects (e.g. herbivory, mechanical stress, etc.) may counteract this balance.

1. Introduction

In the last century, human activities have triggered changes at a global scale that are affecting ecosystems worldwide, with coastal vegetated ecosystems being one of the most threatened (Large, 2009). These ecosystems are expected to come under increased pressure from climate change and direct anthropogenic factors in the next decades, (Nicholls et al., 2007). In coastal vegetated habitats worldwide, seagrasses (i.e. marine flowering plants) form extensive meadows in intertidal and subtidal environments. These habitats are increasingly recognised for their ecological function and provisioning of human services, including nutrient regeneration (Costanza et al., 1997), water quality improvement (Waycott et al., 2005), reduction in human and wildlife pathogens (Lamb et al., 2017; Sullivan et al., 2017), shoreline protection (Bos et al., 2007; Christianen et al., 2013), suitable breeding habitats (including those for economically relevant species; Cullen-Unsworth et al., 2014), biodiversity hotspots (Duffy, 2006; González-Ortiz et al., 2014a) and carbon sequestration (Fourqurean et al., 2012). These keystone habitats thus are considered one of the richest and most relevant ecosystems worldwide (Ruiz-Frau et al., 2017; Short et al., 2011), with high economic value for humans (e.g. Campagne et al., 2014). This importance is recognised worldwide by different legislations and international conventions like the Convention on Biological Diversity (1992) or the European Habitats Directive (92/43/EEC). Favoured by this legislative framework, seagrass habitats have been specifically targeted for conservation and restoration (Green and Short, 2004). Regrettably, the proximity of seagrasses to anthropogenic littoral impacts and their shallow distribution in estuarine and coastal areas have led to widespread seagrass losses, with a global decline of 7% yr^{-1} (Waycott et al., 2009) and almost 14% of all seagrass species currently endangered (Short et al., 2011). Therefore, it is crucial to understand the responses of these ecosystems to multiple co-stressors in order to provide sound advice on managing for possible future trajectories (Brierley and Kingsford, 2009; Hoegh-Guldberg and Bruno, 2010; Unsworth et al., 2014).

Climatic change effects (e.g. increase in temperature, seawater acidification, frequency of storms, sea level rise, etc.) in combination with coastal anthropogenic and natural stressors (e.g. nutrient load, changes in salinity and littoral current, diseases, etc.) act together in coastal areas, and their effects are expected to increase in the near future (Halpern, 2014; Nicholls et al., 2007). Increased CO_2 concentration in the air and subsequent solubility in seawater reduces pH and modifies the balance of the different dissolved carbonate species (Zeebe and Wolf-Gladrow, 2001; Koch et al., 2013). Partial pressure of carbon dioxide in water is raised under such conditions, which can benefit seagrass primary production as seagrass photosynthesis is generally considered to be carbon limited (Beer et al., 1980; Beardall et al., 1998; Beer and Koch, 1996; Invers et al., 2001). Thus, higher CO_2 is predicted

* Corresponding author at: Department of Biology, University of Cadiz, Campus of International Excellence (CEIMAR), 11510, Puerto Real, Cádiz, Spain.
E-mail address: gonzalo.egea@uca.es (L.G. Egea).

https://doi.org/10.1016/j.marpolbul.2018.02.029
Received 24 February 2017; Received in revised form 2 February 2018; Accepted 15 February 2018
Available online 21 February 2018
0025-326X/

to lead to higher photosynthesis, growth rates, biomass (Hall-Spencer et al., 2008; Jiang et al., 2010; Palacios and Zimmerman, 2007; Short and Neckles, 1999; Takahashi et al., 2016; Zimmerman et al., 1997) and internal non-structural carbohydrates (NSC) concentrations (Campbell and Fourqurean, 2013; Egea et al., 2018; Garrard and Beaumont, 2014; Zimmerman et al., 1997), in the absence of other factors limiting the growth (e.g. nutrients, light). However, it is important to note that extrapolating laboratory results to predict long-term responses in seagrasses is not always easy, since some long-term experiments have shown no significant changes in biomass, shoot density and/or growth rates under CO_2 enrichment (Alexandre et al., 2012; Campbell and Fourqurean, 2013; Cox et al., 2016; Palacios and Zimmerman, 2007).

Several studies have highlighted the importance of temperature in the seagrass metabolism and in the maintenance of a positive carbon balance, since warmer temperatures favour photosynthesis and respiration through their effects on kinetic reactions and metabolism (Evans et al., 1986; Pérez and Romero, 1992; Zimmerman et al., 1989). Some previous experiments have demonstrated that warmer temperature may benefit the flowering (Ruiz et al., 2017), growth and biomass of seagrass species (under high saturating light conditions; Bulthuis, 1987), while reducing the reserves of non-structural carbohydrates through enhancing respiration (Hernán et al., 2017). However, other studies have shown negative effects on plants (Collier and Waycott, 2014; Jordà et al., 2012; Moreno-Marin et al., 2018; Repolho et al., 2017). The final effect will depend on the thermal tolerance of a species and its optimal temperature for photosynthesis, respiration, and growth (Bulthuis, 1987; Collier et al., 2011; Masini and Manning, 1997; Short and Neckles, 1999).

In addition to these variables affected by climate change, the current increase in nutrient load in coastal waters has been identified as a key factor that has the potential to negatively impact seagrass meadows (Antón et al., 2011; Burkholder et al., 2007; Cabaço et al., 2008; Hughes et al., 2004). Several reports have indicated that moderate increases in nutrient load may stimulate seagrass production and biomass (Alcoverro et al., 1997; Jiménez-Ramos et al., 2017a; Pérez et al., 1991; Short, 1987; Udy et al., 1999). However, under conditions of high nitrogen availability, direct ammonium toxicity can curtail plant growth, biomass and survival (Brun et al., 2002; van Katwijk et al., 1997). As with temperature, the net outcome will depend on the effects of nutrient load on the photosynthesis rates and non-structural carbohydrate reserves, which are needed for a rapid ammonium assimilation (Brun et al., 2008; Villazán et al., 2013a).

These three factors directly affect photosynthetic rate, plant production, biomass and non-structural carbohydrate reserves. However, while CO_2 enrichment may have either a positive effect or no effect on seagrasses, temperature and nutrient enrichment may cause positive or negative effects. The net response may depend on the species, the physiological status of the plants and, notably, the interaction between these factors. For instance, higher CO_2 may benefit plants subject to higher temperatures because both the higher photosynthetic and respiration rates expected under higher temperature can benefit from elevated CO_2 levels (e.g. reducing the carbon limitation; Ow et al., 2016; Zimmerman et al., 1997), higher levels of non-structural carbohydrates (e.g. needed for respiration processes; Campbell and Fourqurean, 2013) and higher biomass (e.g. more photosynthetic tissues; Jiang et al., 2010; Palacios and Zimmerman, 2007; Russell et al., 2013). In contrast, warmer temperature may have a detrimental effect on plants subjected to ammonium enrichment because of the decrease in non-structural carbohydrate reserves due to enhanced respiration rates, as demonstrated by van Katwijk et al. (1997) and Moreno-Marin et al. (2018). However, CO_2 enrichment may counterbalance this negative interaction to some extent, because of its associated enhanced rates in photosynthetic and higher non-structural carbohydrate reserves, which are known to reduce ammonium toxicity symptoms (Brun et al., 2002, 2008). In addition, higher nutrient levels (mainly nitrogen) may be beneficial under elevated CO_2 levels, since the resulting higher photosynthesis and growth rates increase the demand for nutrients (Coskun et al., 2016; Stitt and Krapp, 1999).

Therefore, while the plant response to a single factor can be well described and predicted, the combination of multiple factors acting together under natural conditions can induce a complex response difficult to predict, as plants may exhibit non-additive responses when exposed to multiple stressors (Gunderson et al., 2016; Moreno-Marin et al., 2018). Non-additive effects may be antagonistic (i.e. the combined effect is less than the expected additive effect) or synergistic (i.e. greater than the expected additive effect). Some previous works have found mainly non-additive responses when using a multifactorial design with some of the aforementioned stressors (warmer temperature, enhanced CO_2, ammonium enrichment) (Brun et al., 2008; Burnell et al., 2013; Collier et al., 2011; De los Santos et al., 2010; Egea et al., 2018; Jiménez-Ramos et al., 2017b; Koch et al., 2013; La Nafie et al., 2012; Lee et al., 2007; Moreno-Marin et al., 2016, 2018; Repolho et al., 2017; Salo and Pedersen, 2014; Villazán et al., 2013a). Therefore, if plants have a non-additive response, predicting the effects of environmental change from single factor experiments may under- or overestimate the combined effect of multiple stressors.

This work aims to study the response of a temperate seagrass (*Cymodocea nodosa*) to the forecasted global change factors (high temperature, CO_2 increase and ammonium enrichment) using a multifactorial mesocosm experiment, testing whether the combined effects of these stressors are additive or non-additive. Based on previous studies, we hypothesize that the combination of the three factors will have a positive effect on plant production and biomass, while non-structural carbohydrates will be reduced because of their depletion by ammonium assimilation and the enhanced respiratory processes promoted by higher temperature. In addition, we predict that most of the factor combinations will produce non-additive responses.

2. Material and methods

2.1. Field plant collection

Individual shoots of *Cymodocea nodosa* (Ucria) Ascherson were randomly collected from a depth of 1–2 m in submerged seagrass meadows at Cadiz Bay (southern Spain, 36°29′19.79″N; 6°15′53.05″E). Healthy looking vertical shoots with intact rhizomes were transported to the laboratory within 2 h of collection in an ice chest. Once in the laboratory, a large pool of experimental shoots were selected bearing similar lengths, numbers of leaves and roots, and they were cleaned of visible epiphytes. They were acclimated for 5 days in aerated water collected from the sampling site under sub-saturating light (ca. 150 μmol photons $m^{-2} s^{-1}$) with a 16:8 h light:dark cycle at 20 °C before they were used in the experiment.

2.2. Mesocosm experiment

The study was conducted in an open-water indoor mesocosm system at the Faculty of Marine and Environmental Sciences of the University of Cadiz during four weeks in November 2013. The plants were allocated to 1.5 L incubation chambers (n = 24) (Fig. 1). In each chamber, about 18–21 individual *C. nodosa* shoots were planted individually by hand while maintaining similar fresh biomass values (FW) in each chamber, which resulted in a total of ca. 500 shoots planted among all chambers. The total fresh weight (FW) of plants (including leaves, rhizome and root biomasses) in each chamber (B_0, FW) was annotated at the beginning of the experiment. Each chamber had been previously filled with 0.5 L of pre-washed sandy sediment that had been sieved (1 mm) to remove fauna and large particles. We ran a full-factorial indoor mesocosm experiment in a temperature-controlled climate room set at 22 °C to test the effects of three factors: warming, acidification and ammonium enrichment in the seagrass *C. nodosa*. We used two temperature levels, control temperature (CT) ca. 22 °C and high

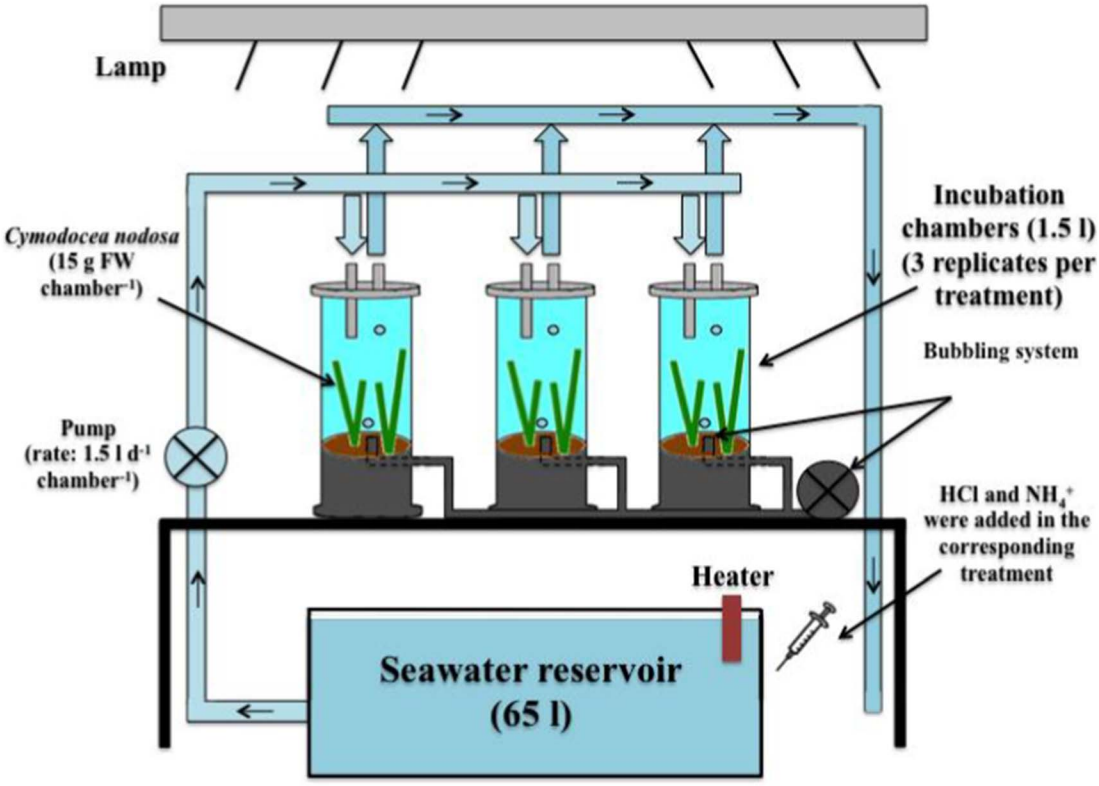

Fig. 1. Simplified diagram of one of the experimental treatments. See detailed description in the text.

Table 1
Water chemical characteristics in each treatment (salinity was 30 psu and light was ca. 325 µmol photons $m^{-2} s^{-1}$). Data are mean ± SE (n = 90). CT = control temperature, HT = High temperature, CpH = Current pH, FpH = Forecasted pH, CNH_4^+ = control NH_4^+, ENH_4^+ = Enrichment NH_4^+.

Treatments			NH_4^+ (µM) *	pH	pCO_2 (ppm)	Temp. (°C)
Temp.	pH	NH_4^+				
HT	FpH	ENH_4^+	32.1 ± 1.4	7.67 ± 0.01	729 ± 12	26.14 ± 0.03
HT	CpH	ENH_4^+	31.4 ± 1.5	8.10 ± 0.02	402 ± 19	26.07 ± 0.03
HT	CpH	CNH_4^+	0	8.10 ± 0.01	412 ± 18	26.08 ± 0.02
HT	FpH	CNH_4^+	0	7.66 ± 0.01	736 ± 12	26.10 ± 0.03
CT	FpH	ENH_4^+	31.8 ± 1.7	7.68 ± 0.01	750 ± 18	21.95 ± 0.08
CT	CpH	ENH_4^+	30.4 ± 1.3	8.13 ± 0.02	424 ± 22	21.94 ± 0.08
CT	CpH	CNH_4^+	0	8.14 ± 0.01	447 ± 21	21.93 ± 0.08
CT	FpH	CNH_4^+	0	7.66 ± 0.01	744 ± 17	21.85 ± 0.05

Notes: All measurements were conducted in the incubation chambers, except for added NH_4^+ (µM) (*), which was conducted in the corresponding reservoirs.

temperature (HT) with seawater heated by 4 °C; two pH levels, current pH (CpH) ca. 8.12, which is equivalent to ca. 415 ppm CO_2, and forecasted pH (FpH) ca. 7.69, equivalent to future conditions of ca. 720 ppm CO_2; and two ammonium levels, control NH_4^+ (CNH_4^+) without NH_4^+ addition and enriched NH_4^+ (ENH_4^+). Nutrient was added to the ENH_4^+ treatment to maintain a constant concentration of ca. 31 µM NH_4^+, which has been used previously in ammonium enrichment experiments (Brun et al., 2002; van Katwijk et al., 1997; Villazán et al., 2016). The factors were manipulated in a fully crossed design, making a total of eight treatments (Table 1). These variables were applied to 65-L seawater reservoirs. Each reservoir, which received sand-filtered seawater from the bay at a rate of 4.5 L d^{-1}, was used to replenish three replicated incubation chambers at a rate of 1.5 L d^{-1} (Fig. 1). The natural seawater used in the reservoirs contained low levels of ammonium (ca. 0.7 µM), nitrate and phosphate (1–2 µM). The incubation chambers were illuminated by lamps with cool fluorescent tubes (T5 High Output Blau Aquaristic aquarium color extreme fluorescents) in a 16:8 h light:dark cycle. This light source created a homogenous field of irradiance in each chamber (325 ± 20 µmol photons $m^{-2} s^{-1}$). Water temperature and pH in the incubation chambers were allowed to fluctuate temporally between light and dark periods to mimic natural conditions in natural seagrass beds. Each incubation chamber (20 $cm^3 h^{-1}$) was individually aerated in order to homogenise the water and reduce the diffusive boundary layer. Once a week, epiphytes growing in the chamber walls were removed and incubation chambers were hazardously reallocated to minimize spatial differences. In addition, all leaves were removed once a week throughout the experimental period and fresh weighed in each chamber. At the end of the experiment (four weeks), all surviving plants from each incubation chamber were harvested and weighed (B_f, FW).

2.3. Temperature, inorganic carbon and ammonium treatments

Temperature and pH levels were manipulated according to the scenario forecasted by the Intergovernmental Panel on Climate Change (IPCC) (Ciais et al., 2013; Prinn et al., 2011). Temperatures were maintained by recirculating water through a heater (Tetra HT 100 W). The pH values in the forecasted pH reservoirs were reached by adding

Table 2
Summary of seawater chemistry in the different treatments. Data are mean ± SE, $n = 9$. Salinity ~ 30 ppt and temperature ~ 21 °C in all treatments. TA = Total alkalinity, pH_T = total pH, DIC = dissolved inorganic carbon. The values for pCO_2 were calculated by the computer programme CO2SYS package (version 2.1) (Lewis and Wallace, 1998). CT = control temperature, HT = High temperature, CpH = Current pH, FpH = Forecasted pH, CNH_4^+ = control NH_4^+, ENH_4^+ = Enrichment NH_4^+.

Incubation treatments			A_T (μmol kg^{-1})	pH_T	DIC (μmol kg^{-1})	pCO_2 (ppm)
Temp.	pH	NH_4^+				
HT	FpH	ENH_4^+	1286 ± 15	7.6 ± 0.01	1238 ± 15	739 ± 14
HT	CpH	ENH_4^+	2485 ± 11	8.1 ± 0.01	2214 ± 12	419 ± 10
HT	CpH	CNH_4^+	2560 ± 22	8.1 ± 0.01	2278 ± 23	423 ± 16
HT	FpH	CNH_4^+	1259 ± 50	7.6 ± 0.01	1210 ± 49	709 ± 19
CT	FpH	ENH_4^+	1345 ± 33	7.6 ± 0.01	1287 ± 30	697 ± 11
CT	CpH	ENH_4^+	2460 ± 20	8.1 ± 0.01	2179 ± 17	396 ± 13
CT	CpH	CNH_4^+	2653 ± 31	8.1 ± 0.01	2358 ± 30	432 ± 11
CT	FpH	CNH_4^+	1329 ± 27	7.6 ± 0.03	1275 ± 22	735 ± 36

small amounts of HCl to the seawater until reaching the pH value necessary for the required CO_2 concentration (ca. 720 ppm total scale) (e.g. Netten et al., 2013). Changes in CO_2 concentration were controlled through daily measures of water pH, salinity and temperature in the incubation chambers. Weekly carbon chemistry parameters were derived using pH (on the total scale), alkalinity, temperature and salinity. Alkalinity samples were collected from each incubation chamber using 250 mL borosilicate bottles, just before sunrise, and a saturated solution of $HgCl_2$ was added following the methods outlined in the DOE handbook (DOE, 1994). Alkalinity was determined using the Gran titration technique with HCl 0.1 N using a Methrom Ion Analysis (tiamo, version 1.2 light with titrando 808, stirrer 801, pH meter 780 and sonda metrohm 6.0262.100 (Metrohm AG CH-9101, Herisau, Switzerland)) following Pérez et al. (2000). Total inorganic carbon (Ci) and partial pressure of CO_2 (pCO_2) were estimated from pH, alkalinity, temperature and salinity data using the CO2SYS package (Lewis and Wallace, 1998), with the K1 and K2 constants from Mehrbach et al. (1973) as modified by Dickson and Millero (1987), and the $KHSO_4$ constant from Dickson (1990). The pH, total alkalinity (TA), temperature, salinity and carbon speciation within the incubation chambers are shown in Table 2. Regarding ammonium concentrations, control NH_4^+ reservoirs were maintained to resemble field conditions. The enriched NH_4^+ reservoirs were manipulated by adding ammonium to the reservoir from a NH_4^+ stock solution every day to keep the concentrations as close as possible to the target concentration (ca. 31 µM NH_4^+). The NH_4^+ addition corresponded to ca. 700 µmol g FW^{-1} d^{-1} in the enriched NH_4^+ treatments. The concentration of ammonium was monitored according to Invers et al. (2004) every two to three days in the chambers and every day in the reservoirs. Water samples were collected right after adding ammonium, although the first ammonium measures in the incubation chambers were taken two days after the start of the experiment.

2.4. Laboratory analysis

After 30 days of culture, only living plants were collected to measure production (net production, leaf loss and gross production rates), non-structural carbohydrates (i.e. sucrose and starch in aboveground and belowground tissues), internal ammonium (aboveground tissues) and C and N content (aboveground tissues). For production measurements, the net production rate (NPR) was obtained by the difference between the fresh biomass at the end of the experiment (B_f) and the initial fresh biomass (B_0), divided by the elapsed time (i.e. 30 days). The leaf loss rate (LLR) was obtained by dividing the accumulated fresh biomass of dead leaves by the experimental time. Finally, gross production rates (GPR) were calculated by adding NPR and LLR, since no plant mortality was found in any of the chambers. The concentration of non-structural carbohydrates (NSC) (i.e. sucrose and starch) was measured in duplicated leaf and rhizome samples from each incubation chamber. Samples were freeze-dried and ground prior to analysis. Total non-structural carbohydrates were measured following Brun et al. (2002). Sugars (sucrose and hexoses) were first solubilized by 4 sequential extractions in 96% (v/v) ethanol at 80 °C for 15 min. The ethanol extracts were evaporated under a stream of air at 40 °C, and the residues were then dissolved in 10 mL of deionized water for analysis. Starch was extracted from the ethanol-insoluble residue by keeping it for 24 h in 1 N NaOH. The sucrose and starch content were determined spectrophotometrically using a resorcinol and anthrone assay with absorbances of 486 and 640 nm, respectively, and sucrose as the standard. NSC plant budget was calculated as the sum of above and belowground sucrose and starch in each plant. For internal ammonium, the intracellular concentrations of NH_4^+ were measured in duplicate leaf samples from each incubation chamber. Samples were rinsed in deionized water and ca. 0.5 g (FW) was ground in 20 mL of boiling deionized water (Dortch et al., 1984). Samples were sonicated for 10 min and then centrifuged for 20 min at 5000*g*. The concentration of NH_4^+ was finally measured in the supernatant according to Bower and Holm-Hansen (1980) and Grasshoff et al. (1983). Total C and N content were determined using duplicate freeze-dried, ground samples of leaves and roots/rhizomes from each aquarium using a Carlo-Erba NA-1500 CHNS analyzer.

2.5. Data and statistical analysis

Prior to any statistical analysis, data were checked for normality (Shapiro-Wilk normality test) and homoscedasticity (Bartlett test of homogeneity of variance test). The effects of single and combined treatment factors (temperature, acidification and ammonium addition) on gross production rate (GPR), leaf loss rate (LLR), net production rates (NPR), non-structural carbohydrates (i.e. the above and below ground sucrose and starch and the NSC plant budget), C and N content and NH_4^+ internal concentrations were tested using a 3-way ANOVA. When significant differences were found, the Tukey post-hoc test was applied to compare both the levels and interaction factors. Data are presented as mean ± SE. The significance level (α) was set at 0.05 in all tests performed.

We tested whether the effects of combined stress imposed by high temperature (HT), forecasted pH (FpH) and enrichment ammonium (ENH_4^+) were additive or non-additive (i.e. synergistic or antagonistic) using relative response ratios (RR) for each variable as the following:

$$RR = (\text{Stress treatment–Non–stressed})/\text{Non} - \text{stressed} \quad (1)$$

where "Stress treatment" is the measured mean response for each stress treatment (i.e. HT, FpH, ENH_4^+ and combinations of these) and "Non-stressed", for the control situation (i.e. the treatment control temperature, current pH and control ammonium). We used an additive null model as the expected additive response (Darling and Côté, 2008):

$$RR_{Additive} = RR_{Stressor\ 1} + RR_{Stressor\ 2} \quad (2)$$

Error terms were calculated separately for each RR, and the

bootstrap procedure was used to estimate the means and confidence intervals (CI) of each response variable (Efron and Tibshirani, 1986). Bootstrap means and confidence intervals were computed by resampling 1500 values among the original data for each parameter using the "bootES" package v1.2 in R software (Gerlanc and Kirby, 2016). Each set of drawn numbers was then combined to estimate relative responses using Eqs. (1) & (2).

We then compared the observed combined response and the expected additive response. If the observed combined response was less than the expected additive response, the effect was classified as antagonistic. If the observed combined response was greater than the expected additive response, the effect was classified as synergistic. If the observed combined response overlapped with the expected additive response, the effect was classified as additive.

Statistical analyses were computed with R 3.0.2 (R Core Team, 2013).

3. Results

3.1. Ammonium concentration in seawater

Generally, the added ammonium was effectively removed by the plants during the experiment under the control temperature and NH_4^+ enrichment treatment combinations (Fig. 2A). However, some ammonium did accumulate in the seawater in the treatment *high temperature + control pH* during the first days of the experiment. The highest accumulation level was recorded in the treatment *high temperature + forecasted pH* during the first two weeks of the experiment (4–6 μM NH_4^+; Fig. 2B).

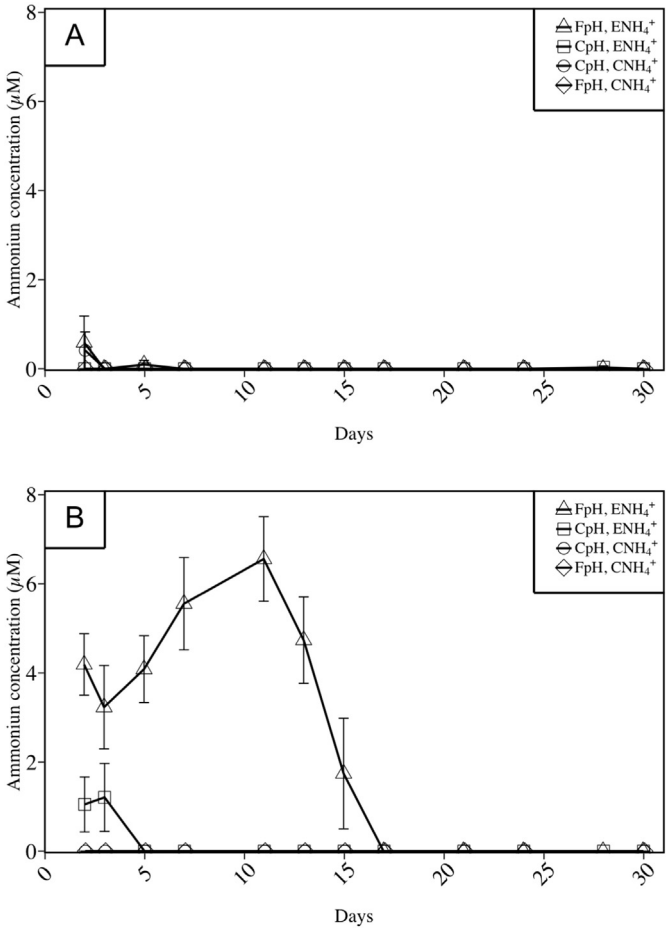

Fig. 2. Ammonium concentrations in seawater under [A] control temperature (CT) and [B] high temperature (HT). CpH = Current pH, FpH = Forecasted pH, CNH_4^+ = control NH_4^+, ENH_4^+ = Enrichment NH_4^+. Data are mean ± SE (n = 3).

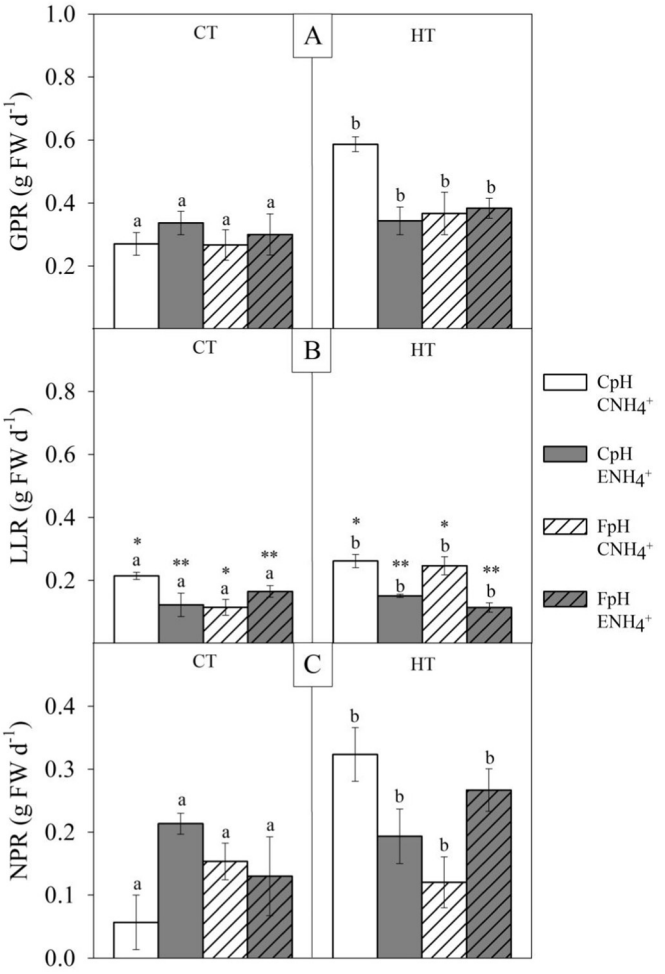

Fig. 3. Effects of temperature (CT vs. HT), pH (CpH vs. FpH) and NH_4^+ (CNH_4^+ vs. ENH_4^+) on [A] Gross Production Rate (GPR), [B] Leaf Loss Rate (LLR) and [C] Net Production Rate (NPR). Letters above the bars represent significant differences in temperature levels; asterisks above the bars represent significant differences in NH_4^+ levels. Data are mean ± SE (n = 3).

3.2. Effects on plant production

No dead plants were detected in any of the chambers, regardless of treatment; thus, mortality rate was zero. Temperature significantly affected GPR. The production in the treatment *high temperature + control* NH_4^+ *+ control pH* was, on average, 1.8 times higher than those in all other treatments (Fig. 3A; Table 3). The combined effect of *high temperature + enrichment* NH_4^+ and the combined effect of *high temperature + enrichment* NH_4^+ *+ forecasted pH* produced significant differences in GPR, mainly due to the effect of temperature. LLR was significantly affected by NH_4^+ enrichment, with enriched being 1.5 times lower than *control* NH_4^+ treatments, on average (Fig. 3B; Table 3). Overall, NPR increased significantly under *high temperature* treatments and also under the combination treatment of the three factors (Fig. 3C; Table 3). Thus, the treatments with higher NPR were *high temperature + control* NH_4^+ *+ control pH* (2.4 times higher than the average of the other treatments) and *high temperature + enrichment* NH_4^+ *+ forecasted pH* (2 times higher than the average of the other treatments). No significant response in any of the three variables was found with forecasted pH as a single factor.

Table 3
Results of the 3-way ANOVA test on treatments (temperature, pH decrease and NH_4^+ addition) and relevant interactions for gross production rate (GPR: g FW d^{-1}), leaf loss rates (LLR: g FW d^{-1}) and net production rates (NPR: g FW d^{-1}).

Variable, factors	df	MS	F	*p*-value
Gross production rate				
Temperature	1	0.09563	14.695	0.001*
pH	1	0.01955	3.004	0.102
NH_4^+	1	0.00657	1.009	0.33
Temperature:pH	1	0.00746	1.146	0.3
Temperature:NH_4^+	1	0.03961	6.086	0.025*
pH:NH_4^+	1	0.01821	2.797	0.114
Temperature:pH:NH_4^+	1	0.0319	4.902	0.042*
Residuals	16	0.00651		
Leaf loss rates				
Temperature	1	0.0092	6.131	0.025*
pH	1	0.004526	3.016	0.102
NH_4^+	1	0.03041	20.264	< 0.001*
Temperature:pH	1	0.00001	0.006	0.937
Temperature:NH_4^+	1	0.015296	10.193	0.005*
pH:NH_4^+	1	0.005443	3.627	0.075
Temperature:pH:NH_4^+	1	0.010164	6.773	0.019*
Residuals	16	0.001501		
Net production rates				
Temperature	1	0.06112	11.683	0.003*
pH	1	0.01127	2.155	0.161
NH_4^+	1	0.0162	3.097	0.097
Temperature:pH	1	0.00306	0.584	0.455
Temperature:NH_4^+	1	0.01203	2.3	0.149
pH:NH_4^+	1	0.0091	1.74	0.206
Temperature:pH:NH_4^+	1	0.0601	11.488	0.004*
Residuals	16	0.00523		

Notes: All data were normally distributed.
* Significance level, $p < 0.05$.

3.3. Effects on non-structural carbohydrates content

Non-structural carbohydrates (NSC) content in both aboveground (leaves) and belowground (rhizomes and roots) tissues were affected by temperature. While there were no significant responses in sucrose content in aboveground tissues among treatments, belowground sucrose content was affected significantly with *high temperature* treatments being, on average, 1.5 times lower than treatments under control temperature (Fig. 4A, B; Table 4). Regarding starch, aboveground tissues were significantly affected by *high temperature* treatments, with these being 1.5 times lower than *control temperature* treatments, on average (Fig. 4C; Table 4). When NSC plant budget was analysed, that is the sum of above and belowground sucrose and starch in each plant, temperature had always a negative significant effects ($p = 0.0179$) alone or when combined with one of the other factors. Although forecasted pH did not yield significant effects on non-structural carbohydrates, there was a weak effect in starch content. Thus, forecasted pH yielded, on average, 1.3 higher contents in aboveground tissues and 0.8 lower contents in belowground ones than under control pH (Fig. 4C, D; Table 4). The combined effect of the three factors caused a significant decrease in starch content, while sucrose usually increased in both tissues (Fig. 4D; Table 4). However, a significant increase in NSC plant budget was recorded when compared to control treatment (236.4 ± 11.1 vs 203.6 mg g DW^{-1}; $p = 0.004$) when the three factors were acting together.

3.4. Effects on total carbon, total nitrogen and ammonium tissue content

Temperature and pH did not affect C content, N content and internal ammonium concentration in aboveground tissues. In contrast, ammonium addition had a significant effect on plant tissue content, resulting in higher average N content (among 14–37%, either in NH_4^+ enrichment treatment alone and in combination with the other factors respect the control treatment; i.e. *control temperature* + *control* NH_4^+ + *current pH*) and higher NH_4^+ internal concentration (among 23–89% in NH_4^+ enrichment treatments combined with the other factors respect the control treatment; i.e. *control temperature* + *control* NH_4^+ + *current pH*) (Fig. 5B, C; Table 5). The combination of factors did not produce significant effects on C and N content. However, it produced significant effects on internal ammonium content as the combination *high temperature* + *enrichment* NH_4^+ effect on internal ammonium was 1.7 times higher than the average of the other treatments (Fig. 5B; Table 5).

3.5. Response ratios

The response ratios for combined treatments were larger than for

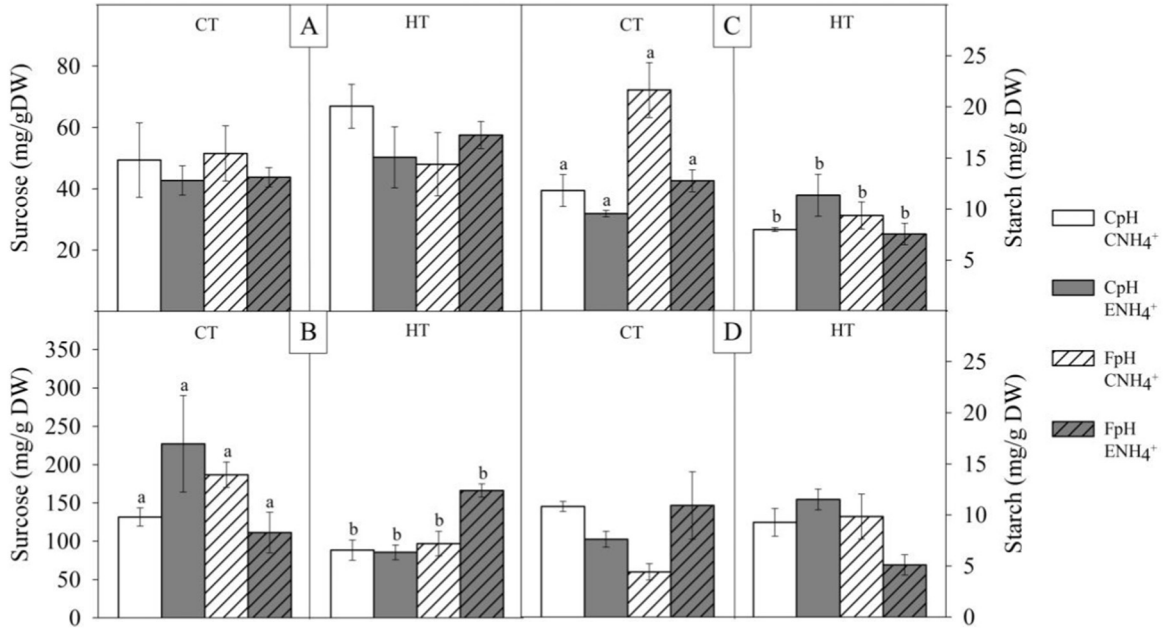

Fig. 4. Effects of temperature (CT vs. HT), pH (CpH vs. FpH) and NH_4^+ (CNH_4^+ vs. ENH_4^+) on [A] aboveground sucrose, [B] belowground sucrose, [C] aboveground starch and [D] belowground starch concentrations. Letters above the bars represent significant differences in temperature levels. Data are mean ± SE (n = 3).

Table 4
Results of the 3-way ANOVA test on treatments (temperature, pH decrease and NH_4^+ addition) and relevant interactions for aboveground sucrose, belowground sucrose, aboveground starch and belowground starch concentrations (mg sucrose or starch g DW^{-1}).

Variable, factors	df	MS	F	*p*-value
Aboveground sucrose				
Temperature	1	469.1	2.328	0.147
pH	1	26.4	0.131	0.722
NH_4^+	1	174.1	0.864	0.366
Temperature:pH	1	82.7	0.411	0.531
Temperature:NH_4^+	1	20.5	0.102	0.754
pH:NH_4^+	1	235	1.167	0.296
Temperature:pH:NH_4^+	1	279.3	1.387	0.256
Residuals	16	201.5		
Belowground sucrose				
Temperature	1	0.8947	11.074	0.004*
pH	1	0.0683	0.846	0.371
NH_4^+	1	0.0753	0.932	0.349
Temperature:pH	1	0.4548	5.629	0.031*
Temperature:NH_4^+	1	0.1512	1.872	0.19
pH:NH_4^+	1	0.0773	0.957	0.343
Temperature:pH:NH_4^+	1	0.9889	12.241	0.003*
Residuals	16	0.0808		
Aboveground starch				
Temperature	1	0.9806	20.042	< 0.001*
pH	1	0.1546	3.16	0.094
NH_4^+	1	0.1419	2.901	0.108
Temperature:pH	1	0.4876	9.967	0.006*
Temperature:NH_4^+	1	0.2455	5.018	0.04*
pH:NH_4^+	1	0.2703	5.524	0.032*
Temperature:pH:NH_4^+	1	0.0154	0.315	0.582
Residuals	16	0.0489		
Belowground starch				
Temperature	1	1.39	0.177	0.679
pH	1	29.97	3.806	0.069
NH_4^+	1	0.25	0.031	0.861
Temperature:pH	1	2.83	0.359	0.557
Temperature:NH_4^+	1	12.64	1.605	0.223
pH:NH_4^+	1	2.79	0.354	0.56
Temperature:pH:NH_4^+	1	104.5	13.269	0.002*
Residuals	16	7.88		

Notes: All data were normally distributed, except for belowground sucrose and aboveground starch to which a natural logarithmic transformation was applied.
* Significance level, $p < 0.05$.

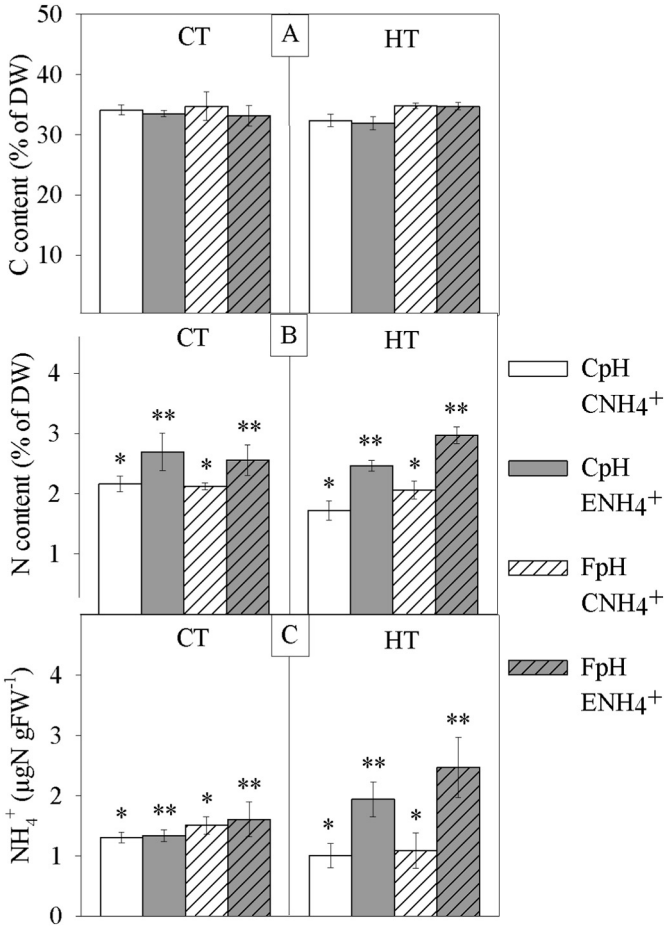

Fig. 5. Effects of temperature (CT vs. HT), pH (CpH vs. FpH) and NH_4^+ (CNH_4^+ vs. ENH_4^+) on [A] C content (% DW), [B] N content (% DW) and [C] NH_4^+ internal concentration (µg N gFW^{-1}). Symbols above the bars represent significant differences in NH_4^+ levels. Data are mean ± SE (n = 3).

the single factor treatments but were rarely significantly different from the corresponding expected additive response ratio as evaluated by the 95% confidence limits (Table 6). Belowground starch and net production rate were the exceptions. In belowground starch, all two-factor treatments exceeded the expected additive effects substantially. In net production rate, the combined effect of all two-factor treatments with high temperature were substantially lower than the expected additive effects.

4. Discussion

Ecological experiments may target the integrated responses of individuals to experimental factors or seek underlying mechanisms to explain such responses (Irschick et al., 2013). In the present study, the response of *Cymodocea nodosa* to the assayed stressors was analysed using a set of response variables that integrated the final response at the plant level (i.e. survival, GPR, NPR and LLR) and some of the main indicators of responses at the physiological level (i.e. non-structural carbohydrates (NSC), internal NH_4^+, C and N content). Plant-level response demonstrated that although all the plants survived during the experimental time, the assayed stressors resulted in large differences in production, depending on the combination of factors. In addition, a wide variety of responses, ranging from additive to non-additive (e.g. antagonistic and synergistic), was recorded when the factors were combined.

4.1. Effects of high temperature alone

When focusing on the response of single factors acting in isolation, temperature had the strongest effect on plant production, causing a significant increase in gross production rate (GPR) and net production rate (NPR). This was probably due to the positive effect of higher temperature on the enzymatic machinery of photosynthesis, as previously demonstrated for *Cymodocea nodosa* (Pérez and Romero, 1992; Terrados and Ros, 1995). The optimum temperature range at which *C. nodosa* maintains its net production is between 10 and 32 °C (Drew, 1978; Pérez and Romero, 1992). Therefore, the positive effect found under high temperature can be attributed to the fact that *C. nodosa* was grown within this optimum range throughout the experimental period. Plants also showed changes in NSC under high temperature conditions, with a lower NSC content in plants exposed to high temperature. As temperature enhances metabolic activity (including respiration), plants may have to use their stored carbohydrates (mainly sucrose) in response to the increase in energy requirement and carbon demand (Burke et al., 1996; Collier et al., 2011; Massa et al., 2009). Therefore, warmer temperature may increase plant production but simultaneously decrease internal carbon reserves. Since internal carbon reserves are essential for plant survival under seasonal fluctuations (e.g. light and temperature; Silva et al., 2013; Soisson et al., 2018), local disturbances (e.g. biomass removal by grazing, sedimentation; Fourqurean et al., 2010; Soisson et al., 2018) and short- and long-term stress events (e.g.

Table 5
Results of the 3-way ANOVA test on treatments (temperature, pH decrease and NH_4^+ addition) and relevant interactions for C content (% DW), N content (% DW) and NH_4^+ internal concentration (μg NH_4^+ gFW^{-1}).

Variable, factors	df	MS	F	*p*-value
Total C content				
Temperature	1	1.07	0.229	0.639
pH	1	11.21	2.402	0.141
NH_4^+	1	2.72	0.583	0.456
Temperature:pH	1	9.43	2.02	0.174
Temperature:NH_4^+	1	1.09	0.234	0.635
pH:NH_4^+	1	0.15	0.032	0.86
Temperature:pH:NH_4^+	1	0.65	0.139	0.715
Residuals	16	4.666		
Total N content				
Temperature	1	0.04	0.415	0.5284
pH	1	0.1667	1.73	0.2069
NH_4^+	1	2.5742	26.722	< 0.001*
Temperature:pH	1	0.3902	4.05	0.0613
Temperature:NH_4^+	1	0.1803	1.871	0.1902
pH:NH_4^+	1	0.0014	0.014	0.9072
Temperature:pH:NH_4^+	1	0.0241	0.25	0.624
Residuals	16	0.0963		
NH_4^+ content				
Temperature	1	2.09E-07	0.96	0.342
pH	1	4.48E-07	2.058	0.171
NH_4^+	1	2.26E-06	10.363	0.005*
Temperature:pH	1	6.700E-0.9	0.031	0.863
Temperature:NH_4^+	1	1.79E-06	8.233	0.011*
pH:NH_4^+	1	1.014E-0.7	0.466	0.505
Temperature:pH:NH_4^+	1	5.23e-0.8	0.24	0.631
Residuals	16	2.178E-0.7		

Notes: All data were normally distributed.
* Significance level, $p < 0.05$.

nutrient enrichment, eutrophication, etc.; Brun et al., 2002, 2003; Moreno-Marin et al., 2018; van Katwijk et al., 1997; Terrados et al., 1999), this reduction in NSC may endanger plant capacity to respond to additional external stressors.

4.2. Effects of NH_4^+ enrichment alone

In our experimental design, ammonium addition can be considered to some extend as a stressor, since its toxicity has been demonstrated for several photosynthetic organisms (reviewed by Britto and Kronzucker, 2002), including seagrasses (Brun et al., 2002, 2008; Moreno-Marín et al., 2016; van Katwijk et al., 1997; Villazán et al., 2013b, 2015). In addition, the internal concentration of ammonium increased under the high temperature treatments, which may be considered as an early symptom of ammonium toxicity (Villazán et al., 2015). However, in this experiment, ammonium addition alone or in combination did not have a negative effect at the whole plant level (i.e. survival or plant production rates), since we even found a slight stimulation of net production when compared with the control treatment (Fig. 3C). This can be explained if we take into account that initial internal nitrogen was 1.1 ± 0.13% ($n = 3$; data not shown) and that in control plants, nitrogen content at the end of the experiment was 2.16 ± 0.13 (Fig. 5B); thus, plants were nutrient limited (Duarte, 1990). The high levels of irradiance in the incubation chambers, which may provide enough energy and carbon from photosynthesis to undertake ammonium assimilation, may also help to explain these results (Brun et al., 2002, 2008; Moreno-Marín et al., 2016; Villazán et al., 2015). In addition, the concentration at which ammonium starts to produce negative effects on seagrasses shows inter- and intra-specific variability (Brun et al., 2002, 2008; Quark et al., 2016; van der Heide et al., 2008; van Katwijk et al., 1997). In the case of *C. nodosa*, the threshold seems to be higher than the concentration used in this experiment, which has been demonstrated to produce negative effects in other seagrass species (e.g. *Zostera noltei* and *Z. marina*; Brun et al., 2002; van der Heide et al., 2008; van Katwijk et al., 1997; Villazán et al., 2013a, 2013b).

Accumulation of ammonium in seawater was found in the combined treatments, especially at high temperature conditions, under which plants showed lower capacity to reduce the added ammonium in the early days of the incubation (Fig. 2). This may be a sign of increased vulnerability against ammonium mainly due to high temperature as explained above. However, this accumulated ammonium in the water disappeared after two weeks under these treatments, which can be explained using two different but complementary processes. On one hand, the growth of the plants during the experimental period (0.36 ± 0.02 g FW d^{-1}) may have enhanced the ammonium uptake capacity in the chambers. On the other hand, an increase in the sediment microbial benthic community throughout the experimental period (as a consequence of experimental conditions and boosted by the factors used; i.e. temperature and/or nutrients; Nydahl et al., 2013; Sarmento et al., 2010), may also considerably reduce ammonium concentrations in the water column (Moreno-Marín et al., 2016). However, as sediment was sieved and cleaned before starting the experiment, the development of this microbial benthic community in the sediment takes few weeks (García-Robledo et al., 2016).

4.3. Effect of acidification alone

Acidification increased CO_2 availability, which is known to considerably improve photosynthesis in this species (Beer et al., 1980; Invers et al., 1997, 1999, 2001), and also reduce the time of saturating light needed to maintain a positive whole-plant carbon balance (Zimmerman et al., 1997). However, this did not translate into a better performance at the whole plant level (i.e. production rates) in our experiment, which is in agreement with some previous studies on seagrasses (e.g. Alexandre et al., 2012; Campbell and Fourqurean, 2013; Cox et al., 2016; Martínez-Crego et al., 2014; Palacios and Zimmerman, 2007; Schwarz et al., 2000) and may be related to the nutrient limitation suggested by our data. Since photosynthesis and production may be favoured under high CO_2 levels, nutrients demand (mainly nitrogen) also increase. If nutrients are in low supply, photosynthesis may be reduced in a process known as acclimation of photosynthesis to elevated CO_2 concentrations (Coskun et al., 2014; Stitt and Krapp, 1999). Therefore, this initial benefit of CO_2 increase (improvement of photosynthetic rates, reduction in light requirements, etc.) may vanish at the whole plant level under nutrient limitation. Although we did not detect a significant effect at the whole plant level, at the physiological level, an increase of starch in aboveground tissues was found at expenses of a decrease in starch in belowground tissues (although it does not lead to being significant).

4.4. Effect of combined multiple stressors

Even though the underlying mechanistic basis of seagrass response to individual factors can be explored and well described, the complexity of nature makes the final response difficult to predict. To improve predictions, studies are required that explore the effects of the factors in situ over the long-term (Campbell and Fourqurean, 2013; Cox et al., 2016; Takahashi et al., 2016) and also address factors in multifactorial designs. The effect of combined multiple stressors are often assumed to be accumulative (Halpern et al., 2007); however, as shown by this study, a large fraction of the responses at the whole plant and physiological levels were additive, but others were antagonistic or synergistic (Table 6), which is consistent with previous studies of combined multiple stressors on seagrasses (e.g. Moreno-Marin et al., 2018; Villazán et al., 2016). For instance, all two-factors combined responses were substantially larger than the sum of their individual responses for belowground starch. This underlines that simultaneous exposure to high temperature, NH_4^+ enrichment and pH decrease have a synergistic effect on starch reserves. In contrast, net production rate under NH_4^+

Table 6
Relative response ratios (Eq. (1)) on Gross Production Rate (GPR), Leaf Loss Rate (LLR), Net Production Rate (NPR), aboveground sucrose (AG sucrose), belowground sucrose (BG sucrose), aboveground starch (AG starch), belowground starch (BG starch), C content, N content and NH_4^+ internal concentration in *Cymodocea nodosa* plants when exposed to high temperature (HT) alone, forecasted pH (FpH) alone, enrichment NH_4^+ (ENH_4^+) alone and when these single factors were combined. The expected additive response is the null model to which the combined response was tested. Values shown are adjusted bootstrap means and 95% confidence interval (in brackets). Add = Additive, Antag = Antagonistic, Synerg = Synergistic.

	HT alone	FpH alone	ENH_4^+ alone	Expected additive response (HT + FpH)	Observed combined response (HT + FpH)	Effect	Expected additive response (HT + ENH_4^+)	Observed combined response (HT + NH_4^+)	Effect	Expected additive response (FpH + ENH_4^+)	Observed combined response (FpH + ENH_4^+)	Effect	Expected additive response (All)	Observed combined response (All)	Effect
GPR	+117% (93, 143)	−2% (−44, 31)	+24% (−4, 64)	+115% (49, 174)	+35% (−16, 75)	Add.	+141% (89, 207)	+27% (−3, 63)	Add.	+23% (−48, 95)	+10% (−29, 58)	Add.	+139% (45, 238)	+40% (13, 73)	Add.
LLR	+22% (5, 40)	−47% (−67, −25)	−43% (−73, −17)	−25% (−62, 15)	+15% (−14, 33)	Add.	−21% (−68, 23)	−30% (−38, −20)	Add.	−90% (−140, −42)	−23% (−40, −8)	Synerg.	−68% (−135, −2)	−47% (−63, −35)	Add.
NPR	+475% (278, 630)	+170% (14, 308)	+280% (149, 400)	+645% (292, 939)	+112% (−64, 278)	Antag.	+755% (427, 1031)	+242% (78, 406)	Antag.	+449% (163, 708)	+135% (−71, 349)	Add.	+925% (441, 1339)	+371% (186, 515)	Add.
AG sucrose	+36% (−27, 72)	+4% (−58, 44)	−14% (−70, 18)	+40% (−86, 116)	−3% (−51, 45)	Add.	+22% (−98, 90)	+2% (−68, 45)	Add.	−9% (−129, 63)	−11% (−66, 17)	Add.	+26% (−156, 134)	+17% (−36, 48)	Add.
BG sucrose	−33% (−60, −13)	+42% (20, 67)	+73% (0, 160)	+9% (−41, 54)	−26% (−48, −2)	Add.	+40% (−61, 147)	−35% (−55, −18)	Add.	+115% (19, 227)	−15% (−47, 17)	Antag.	+82% (−41, 214)	+26% (3, 41)	Add.
AG starch	−32% (−48, −6)	+83% (27, 117)	−19% (−36, 7)	+51% (−21, 110)	−21% (−45, 6)	Add.	−52% (−84, 0)	−4% (−48, 27)	Add.	+64% (−9, 123)	+8% (−14, 34)	Add.	+32% (−57, 117)	−36% (−58, −8)	Add.
BG starch	−14% (−38, 4)	−59% (−75, −47)	−30% (−41, −14)	−73% (−113, −54)	−9% (−50, 16)	Synerg.	−44% (−79, −10)	+6% (−10, 22)	Synerg.	−89% (−116, −61)	+1% (−53, 41)	Synerg.	−103% (−154, −57)	−53% (−70, −38)	Add.
C content	−5.2% (−12, 0)	+1.8% (−12, 12)	−1.8% (−6, 2)	−3.4% (−24, 12)	+2% (−3, 6)	Add.	−7% (−18, 3)	−6.4% (−15, −1)	Add.	0% (−18, 14)	−2.9% (−15, 4)	Add.	−5.2% (−30, 14)	+1.8% (−3, 6)	Add.
N content	−20.5% (−35, −6)	−1.7% (−15, 6)	+24.6% (−4, 46)	−22.2% (−50, 0)	−4.6% (−20, 9)	Add.	+4.1% (−39, 40)	+14.1% (0, 24)	Add.	+22.9% (−19, 53)	+18.3% (−6, 35)	Add.	+2.4% (−54, 46)	+37.3% (22, 50)	Add.
NH_4^+ internal	−23% (−45, 7)	+15.7% (−5, 36)	+2.4% (−15, 19)	−7.3% (−49, 43)	−16.7% (−51, 27)	Add.	−20.6% (−62, 20)	+49% (20, 93)	Synerg.	+18.1% (−19, 55)	+23.5% (−10, 61)	Add.	−4.9% (−64, 61)	+89.5% (40, 168)	Add.

enrichment combined with one of the other assayed stressors was substantially lower than the sum of the two individual responses, underlining that simultaneous exposure to NH_4^+ enrichment with high temperature or pH decrease have an antagonistic effect on plant level responses. The occurrence of synergistic and antagonistic responses to multiple stressors should therefore be taken into account in the future management of seagrass communities.

The interaction of all three factors yielded one of the highest increases in net production rate. High temperature is probably the main driver of this increase, as we noted above, but contrary to expectation, there was no significant decrease in sugar content when combined with the other two factors (even an increase in belowground sucrose was recorded). The CO_2 increase may have buffered the NSC decrease derived of high temperature, which has been also recorded in previous studies in seagrasses when plants were subjecting to additional stress factors draining carbon reserves (Burke et al., 1996; Collier et al., 2011; Massa et al., 2009). In addition, this higher NSC levels may counterbalance the negative effects derived of ammonium assimilation (Brun et al., 2008; Villazán et al., 2015). Thus, these results demonstrate that the combined effect of the three factors triggered a positive response of *Cymodocea nodosa*, improving their production and fitness enhancing their NSC concentrations, which may in addition improve plant resistance to other stressors. Based on these results, it seems that climate change and to some extent nutrient enrichment in coastal areas may not be so detrimental to seagrasses as previously believed (Orth et al., 2006), and may even benefit *C. nodosa* productivity and resistance in the future under the conditions studied here. In this regard, seagrass meadows are natural hot spots to fight against climate change, as they may benefit from these changes and have large potential for uptake of excess anthropogenic CO_2 (Duarte and Cebrián, 1996; Kennedy et al., 2010; Russell et al., 2013). Therefore, conservation management to protect and increase seagrass meadows is one potential solution to the global problems we face.

The studies of the effects of climate change on coastal ecosystems is very complex given the large number of affected variables and all their possible interactions. This experiment has focused on the effect of the main variables related to climate change along with nutrient enrichment in *Cymodocea nodosa*. However, the results may not apply to other seagrass species. For example, Martínez-Crego et al. (2014) found a weak response of *Zostera noltei* plants to the combined effect of acidification and ammonium enrichment. Moreover, species that live close to their upper limit of temperature tolerance will probably be affected in a negative way by climate change (especially those that live in tropical zones). In addition, although future environmental conditions may be favourable for this species in temperate latitudes, other variables not studied here could affect the global response of this species. Examples of possible variables include decreases in light (as a result of sea level rise and more favourable conditions for the growth of algae and epiphytes that compete with plants; Apostolaki et al., 2011; Martínez-Crego et al., 2014; Ralph et al., 2007), seagrass structural damage (as a result of more frequent storm events; Campbell and McKenzie, 2004; Rasheeh et al., 2014), increase in hydrodynamic stress (Egea et al., 2018; González-Ortiz et al., 2014b), etc. On the other hand, the extrapolation of our experimental results to natural conditions must be taken with caution, since our experimental design (and some of the aforementioned studies) only encompassed an isolated plant species and was not applied to communities or ecosystems, as communities may buffer or strengthen the individual responses of a species (Cox et al., 2016; Burnell et al., 2013; Palacios and Zimmerman, 2007). For instance, higher CO_2 levels may reduce the synthesis of some natural products (i.e. phenolic compounds; Arnold et al., 2012; Jiménez-Ramos et al., 2017b), which may increase the palatability of the tissues and increase vulnerability against herbivores, which in turn may shift the initially positive effect on plants to a negative balance if extra consumption surpasses the increase in growth (Jiménez-Ramos et al., 2017b). Moreover, changes in leaf width and thickness have also been recorded in response to CO_2 enrichment (Cox et al., 2016). Both parameters are essential for the biomechanical design of seagrass leaves (De los Santos et al., 2016), which may finally affect the chances of mechanical failure of leaves and/or being consumed by herbivores (De los Santos et al., 2012; Jiménez-Ramos et al., 2017b; Tomas et al., 2015). Hence, more studies are necessary that allow us to delve deep into how these keystone ecosystems will respond in the future in order to properly manage them.

5. Conclusions

Our study shows that although some of the environmental factors studied in this experiment may produce a limited response in *Cymodocea nodosa* when acting alone (CO_2 increase and NH_4^+ enrichment), the combined effect of the three factors triggered a positive response of this seagrass specie. Overall productivity was improved in this species, as were NSC concentrations, which may improve plant resistance to other stressors. In this case, we predict a positive response of *C. nodosa* to the forecasted future conditions of warmer temperature, NH_4^+ enrichment and CO_2 increase as their productivity was enhanced without decreasing non-structural carbohydrates reserves, which are essential when environmental conditions become more stressful. Even though we found a positive effect, it is important to keep in mind that extrapolating these results to in situ conditions must be done with caution, since complex relationships in the ecosystem and other indirect effects may hamper this initially beneficial effect. Overall, this research also highlights the importance of studying environmental factors that interact under natural conditions using a multifactorial approach, as the occurrence of non-additive responses to multiple stressors should be taken into account to yield more realistic predictions of the possible effects of global change and anthropogenic impacts on seagrass ecosystems.

Acknowledgments

This work was supported by the Excelence Project of the Junta Andalucia RNM-P12-3020 (PRODESCA), the Spanish national project CTM2011-24482 (SEA-LIVE), and the Spanish Ministry of Education [FPU12/05055 grant awarded to L.G. Egea]. We thank N. Garzón (CACYTMAR) for laboratory assistance.

References

Alcoverro, T., Romero, J., Duarte, C.M., Lopez, N.I., 1997. Spatial and temporal variations in nutrient limitation of seagrass *Posidonia oceanica* growth in the NW Mediterranean. Mar. Ecol. Prog. Ser. 146, 155–161. http://dx.doi.org/10.3354/meps146155.

Alexandre, A., Silva, J., Buapet, P., Björk, M., Santos, R., 2012. Effects of CO_2 enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*. Ecol. Evol. 2, 2625–2635.

Antón, A., Cebrian, J., Heck, K.L., Duarte, C.M., Sheehan, K.L., Miller, M.-E.C., Foster, C.D., 2011. Decoupled effects (positive to negative) of nutrient enrichment on ecosystem services. Ecol. Appl. 21, 991–1009. http://dx.doi.org/10.1890/09-0841.1.

Apostolaki, E.T., Holmer, M., Marbà, N., Karakassis, I., 2011. Epiphyte dynamics and carbon metabolism in a nutrient enriched Mediterranean seagrass (*Posidonia oceanica*) ecosystem. J. Sea Res. 66, 135–142.

Arnold, T., Mealey, C., Leahey, H., Miller, A.W., Hall-Spencer, J.M., Milazzo, M., Maers, K., 2012. Ocean acidification and the loss of phenolic substances in marine plants. PLoS One 7 (4), e35107.

Beardall, J., Beer, S., Raven, J.A., 1998. Biodiversity of marine plants in an era of climate change: some predictions based on physiological performance. Bot. Mar. 41, 113–123. http://dx.doi.org/10.1515/botm.1998.41.1-6.113.

Beer, S., Koch, E., 1996. Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. Mar. Ecol. Prog. Ser. 141, 199–204. http://dx.doi.org/10.3354/meps141199.

Beer, S., Shomer-Ilan, A., Waisel, Y., 1980. Carbon metabolism in seagrasses. J. Exp. Bot. 31, 1019–1026. http://dx.doi.org/10.1093/jxb/31.4.1019.

Bos, A.R., Bouma, T.J., de Kort, G.L.J., van Katwijk, M.M., 2007. Ecosystem engineering by annual intertidal seagrass beds: sediment accretion and modification. Estuar. Coast. Shelf Sci. 74, 344–348.

Bower, C.E., Holm-Hansen, T., 1980. A salicylate–hypochlorite method for determining ammonia in seawater. Can. J. Fish. Aquat. Sci. 37, 794–798. http://dx.doi.org/10.1139/f80-106.

Brierley, A.S., Kingsford, M.J., 2009. Impacts of climate change on marine organisms and ecosystems. Curr. Biol. 19, R602–R614. http://dx.doi.org/10.1016/j.cub.2009.05.046.
Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant Physiol. 159, 567–584.
Brun, F.G., Hernández, I., Vergara, J., Peralta, G., Pérez-Lloréns, J., 2002. Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. Mar. Ecol. Prog. Ser. 225, 177–187. http://dx.doi.org/10.3354/meps225177.
Brun, F.G., Vergara, J.J., Navarro, G., Hernández, I., Pérez-Lloréns, J.L., 2003. Effect of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii*. Mar. Ecol. Prog. Ser. 265, 85–96. http://dx.doi.org/10.3354/meps265085.
Brun, F.G., Olivé, I., Malta, E., Vergara, J., Hernández, I., Pérez-Lloréns, J., 2008. Increased vulnerability of *Zostera noltii* to stress caused by low light and elevated ammonium levels under phosphate deficiency. Mar. Ecol. Prog. Ser. 365, 67–75. http://dx.doi.org/10.3354/meps07512.
Bulthuis, D.A., 1987. Effects of temperature on photosynthesis and growth of seagrasses. Aquat. Bot. 27, 27–40. http://dx.doi.org/10.1016/0304-3770(87)90084-2.
Burke, M.K., Dennison, W.C., Moore, K.A., 1996. Non-structural carbohydrate reserves of eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 137, 195–201.
Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Biol. Ecol. 350, 46–72. http://dx.doi.org/10.1016/j.jembe.2007.06.024.
Burnell, O.W., Russell, B.D., Irving, A.D., Connell, S.D., 2013. Eutrophication offsets increased sea urchin grazing on seagrass caused by ocean warming and acidification. Mar. Ecol. Prog. Ser. 485, 37–46.
Cabaço, S., Machías, R., Vieira, V., Santos, R., 2008. Impacts of urban wastewater discharge on seagrass meadows (*Zostera noltii*). Estuar. Coast. Shelf Sci. 78, 1–13. http://dx.doi.org/10.1016/j.ecss.2007.11.005.
Campagne, C.S., Salles, J.M., Boissery, P., Deter, J., 2014. The seagrass *Posidonia oceanica*: ecosystem services identification and economic evaluation of goods and benefits. Mar. Pollut. Bull. 97, 391–400. http://dx.doi.org/10.1016/j.marpolbul.2015.05.061.
Campbell, J.E., Fourqurean, J.W., 2013. Effects of *in situ* CO_2 enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. Mar. Biol. 160, 1465–1475.
Campbell, S.J., McKenzie, L.J., 2004. Flood related loss and recovery of intertidal seagrass meadows in southern Queensland, Australia. Estuar. Coast. Shelf Sci. 60, 477–490.
Christianen, M.J.A., Van Belzen, J., Herman, P.M.J., van Katwijk, M.M., Lamers, L.P.M., Van Leent, P.J.M., Bouma, T.J., 2013. Low-canopy seagrass beds still provide important coastal protection services. PLoS One 8, e62413.
Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R.B., Piao, S., Thornton, P., France, P.C., Willem, J., Friedlingstein, P., Munhoven, G., 2013. Carbon and other biogeochemical cycles. In: Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
Collier, C.J., Waycott, M., 2014. Temperature extremes reduce seagrass growth and induce mortality. Mar. Pollut. Bull. 83, 483–490.
Collier, C.J., Uthicke, S., Waycott, M., 2011. Thermal tolerance of two seagrass species at contrasting light levels: implications for future distribution in the great barrier reef. Limnol. Oceanogr. 56, 2200–2210.
Convention on Biological Diversity, 1992. https://www.cbd.int/cop10/.
Coskun, D., Britto, D.T., Hamam, A.M., Kronzucker, H.J., 2014. Measuring fluxes of mineral nutrients and toxicants in plants with radioactive tracers. J. Vis. Exp. 90, e51877.
Coskun, D., Britto, D.T., Kronzucker, H.J., 2016. Nutrient constraints on terrestrial carbon fixation: the role of nitrogen. J. Plant Physiol. 203, 95–109.
Costanza, R., Arge, R., De Groot, R., Farberk, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Suttonkk, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.
Cox, T.E., Gazeau, F., Alliouane, S., Hendriks, I.E., Mahaeck, P., Le Fur, A., Gattuso, J.P., 2016. Effects of in situ CO_2 enrichment on structural characteristics, photosynthesis, and growth of the Mediterranean seagrass *Posidonia oceanica*. Biogeosciences 13, 2179–2194.
Cullen-Unsworth, L.C., Nordlund, L.M., Paadock, J., Baker, S., McKenzie, L.J., Unsworth, R.K.F., 2014. Seagrass meadows globally as a coupled social-ecological system: implications for human wellbeing. Mar. Pollut. Bull. 83, 387–397.
Darling, E.S., Côté, I.M., 2008. Quantifying the evidence for ecological synergies. Ecol. Lett. 11, 1278−1286.
De los Santos, C., Brun, F.G., Bouma, T.J., Vergara, J.J., Pérez-Lloréns, J.L., 2010. Acclimation of seagrass *Zostera noltii* to co-occurring hydrodynamic and light stresses. Mar. Ecol. Progr. Ser. 398, 127–135.
De los Santos, C.B., Brun, F.G., Onoda, Y., Cambridge, M.L., Bouma, T.J., Vergara, J.J., Pérez-Lloréns, J.L., 2012. Leaf-fracture properties correlated with nutritional traits in nine Australian seagrass species:implications for susceptibility to hebivory. Mar. Ecol. Prog. Ser. 458, 89–102.
De los Santos, C.B., Onoda, Y., Vergara, J.J., Pérez-Lloréns, J.L., Bouma, T.J., La Nafie, Y.A., Cambridge, M.L., Brun, F.G., 2016. A comprehensive analysis of mechanical and morphological traits in temperate and tropical seagrass species. Mar. Ecol. Prog. Ser. 551, 81–94.
Dickson, A.G., 1990. Standard potential of the reaction – AgCl(s) + 1/2 H2(g) = Ag (s) + HCl(aq) and the standard acidicity constant of the ion HSO4− in synthetic seawater from 273.15-K to 318.15-K. J. Chem. Thermodyn. http://dx.doi.org/10.1016/0021-9614(90)90074-Z.
Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res. Part A 34, 1733–1743. http://dx.doi.org/10.1016/0198-0149(87)90021-5.
DOE, 1994. Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water; Version 2. Dickson, A.G., Goyet, C., (ORNL/CDIAC-74).
Dortch, Q., Clayton, J.R., Thoresen, S.S., Ahmed, S.I., 1984. Species differences in accumulation of nitrogen pools in phytoplankton. Mar. Biol. 81, 237–250. http://dx.doi.org/10.1007/BF00393218.
Drew, E.A., 1978. Factors affecting photosynthesis and its seasonal variation in the seagrasses *Cymodocea nodosa* (Ucria) archers. and *Posidonia oceanica* (L.) Delile in the Mediterranean. J. Exp. Mar. Biol. Ecol. 31, 173–194.
Duarte, C.M., 1990. Seagrass nutrient content. Mar. Ecol. Prog. Ser. 67, 201–207. http://dx.doi.org/10.3354/meps067201.
Duarte, C.M., Cebrián, J., 1996. The fate of marine autotrophic production. Limnol. Oceanogr. 41, 1758–1766. http://dx.doi.org/10.4319/lo.1996.41.8.1758.
Duffy, J.E., 2006. Biodiversity and the fuctioning of seagrass ecosystems. Mar. Ecol. Prog. Ser. 311, 233–250.
Efron, B., Tibshirani, R., 1986. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat. Sci. 1, 54–75.
Egea, L.G., Jiménez-Ramos, R., Bouma, T.J., Brun, F.G., 2018. Effects of ocean acidification and hydrodynamic conditions on carbon metabolism and dissolved organic carbon (DOC) fluxes in seagrass populations. Plos One 13, e0192402.
Evans, A.S., Webb, K.L., Penhale, P.A., 1986. Photosynthetic temperature acclimation in two coexisting seagrasses, *Zostera marina* L. and *Ruppia maritima* L. Aquat. Bot. 24, 185–197. http://dx.doi.org/10.1016/0304-3770(86)90095-1.
Fourqurean, J.W., Manuel, S., Coates, K.A., Kenworthy, W.J., Smith, S.R., 2010. Effects of excluding sea turtle herbivores from a seagrass bed: overgrazing may led to loss of seagrass meadows in Bermuda. Mar. Ecol. Prog. Ser. 419, 223–232.
Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., Serrano, O., 2012. Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505–509.
García-Robledo, E., Bohorquez, J., Corzo, A., Jiménez-Arias, J.L., Papaspyrou, S., 2016. Dynamics of inorganic nutrients in intertidal sediments: porewater, exchangeable, and intracellular pools. Front. Microbiol. 26. http://dx.doi.org/10.3389/fmicb.2016.00761.
Garrard, S.L., Beaumont, N.J., 2014. The effect of ocean acidification on carbon storage and sequestration in seagrass beds; a global and UK context. Mar. Pollut. Bull. 86, 138–146. http://dx.doi.org/10.1016/j.marpolbul.2014.07.032.
Gerlanc, D., Kirby, K.N., 2016. bootES (Version 1.2) (Retrieved from). https://cran.r-project.org/web/packages/bootES/bootES.pdf.
González-Ortiz, V., Alcazar, P., Vergara, J.J., Pérez-Lloréns, J.L., Brun, F.G., 2014a. Effects of two antagonistic ecosystem engineers on infaunal diversity. Estuar. Coast. Shelf Sci. 39, 20–26.
González-Ortiz, V., Egea, L.G., Jiménez-Ramos, R., Moreno-Marín, F., Pérez-Lloréns, J.L., Bouma, T.J., Brun, F.G., 2014b. Interactions between seagrass complexity, hydrodynamic flow and biomixing alter food availability for associated filter-feeding organisms. PLoS One 9 (8), e104949.
Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of Sea-water Analysis, 2nd edn. Verlag Chemie, Weinheim.
Green, E.P., Short, F., 2004. World atlas of seagrasses. Bot. Mar. 47, 331. http://dx.doi.org/10.1515/BOT.2004.029.
Gunderson, A.R., Armstrong, E.J., Stillman, J.H., 2016. Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. Annu. Rev. Mar. Sci. 8. http://dx.doi.org/10.1146/annurev-marine-122414-033953.
Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D., Buia, M.C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454, 96–99. http://dx.doi.org/10.1038/nature07051.
Halpern, B.S., 2014. A global map of human impact on marine ecosystems. Science 948. http://dx.doi.org/10.1126/science.1149345.
Halpern, B.S., Selkoe, K., Micheli, F., Kappel, C., 2007. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conserv. Biol. 21, 1301–1315.
van der Heide, T., Smolders, A.J.P., Rijkens, B.G.A., Van Nes, E.H., Van Katwijk, M.M., Roelofs, J.G.M., 2008. Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. Oecologia 158, 411–419.
Hernán, G., Ortega, M.J., Gandara, A.M., Castejón, I., Terrados, J., Tomas, F., 2017. Future warmer seas: increased stress and susceptibility tograzing in seedlings of a marine habitat-forming species. Glob. Chang. Biol. 23, 4530–4543.
Hoegh-Guldberg, O., Bruno, J.F., 2010. The impact of climate change on the world's marine ecosystems. Science 328, 1523–1528. http://dx.doi.org/10.1126/science.1189930.
Hughes, A.R., Bando, K.J., Rodriguez, L.F., Williams, S.L., 2004. Relative effects of grazers and nutrients on seagrasses: a meta-analysis approach. Mar. Ecol. Prog. Ser. 282, 87–99. http://dx.doi.org/10.3354/meps282087.
Invers, O., Romero, J., Pérez, M., 1997. Effects of pH on seagrass photosynthesis: a laboratory and field assessment. Aquat. Bot. 59, 185–194.
Invers, O., Perez, M., Romero, J., 1999. Bicarbonate utilization in seagrass photosynthesis: role of carbonic anhydrase in *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson. J. Exp. Mar. Biol. Ecol. 235, 125–133.
Invers, O., Zimmerman, R.C., Alberte, R.S., Pérez, M., Romero, J., 2001. Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. J. Exp. Mar. Biol. Ecol. 265, 203–217.
Invers, O., Kraemer, G.P., Pérez, M., Romero, J., 2004. Effects of nitrogen addition on

nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. J. Exp. Mar. Biol. Ecol. 303, 97–114. http://dx.doi.org/10.1016/j.jembe.2003.11.005.
Irschick, D.J., Fox, C., Thompson, K., Knapp, A., Baker, L., Meyer, J., 2013. Funcitonal ecology: integrative reseach in the moder age of ecology. Funct. Ecol. 27, 1–4.
Jiang, Z.J., Huang, X.-P., Zhang, J.-P., 2010. Effects of CO_2 enrichment on photosynthesis, growth, and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. J. Integr. Plant Biol. 52, 904–913.
Jiménez-Ramos, R., Mancilla, M., Villazán, B., Egea, L.G., González-Ortiz, V., Vergara, J.J., Pérez-Lloréns, J.L., Brun, F.G., 2017a. Resistance to nutrient enrichment varies among components in the *Cymodocea nodosa* community. J. Exp. Mar. Biol. Ecol. 497, 41–49. http://dx.doi.org/10.1016/j.jembe.2017.09.008.
Jiménez-Ramos, R., Egea, L.G., Ortega, M.J., Hernández, I., Vergara, J.J., Brun, F.G., 2017b. Global and local disturbances interact to modify seagrass palatability. PLoS One 12, e0183256. http://dx.doi.org/10.1371/journal.pone.0183256.
Jordà, G., Marbà, N., Duarte, C.M., 2012. Mediterranean seagrass vulnerable to regional climate warming. Nat. Clim. Chang. 2, 821–824.
van Katwijk, M.M., Vergeer, L.H.T., Schmitz, G.H.W., Roelofs, J.G.M., 1997. Ammonium toxicity in eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 157, 159–173. http://dx.doi.org/10.3354/meps157159.
Kennedy, H., Beggins, J., Duarte, C.M., Fourqurean, J.W., Holmer, M., Marbá, N., Middelburg, J.J., 2010. Seagrass sediments as a global carbon sink: isotopic constraints. Glob. Biogeochem. Cycles 24, 1–8. http://dx.doi.org/10.1029/2010GB003848.
Koch, M., Bowes, G., Ross, C., Zhang, X.H., 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob. Chang. Biol. 19, 103–132. http://dx.doi.org/10.1111/j.1365-2486.2012.02791.x.
La Nafie, Y.A., De los Santos, C.B., Brun, F.G., van Katwijk, M.M., Bouma, T.J., 2012. Waves and high nutrient loads jointly decrease survival and separately affect morphological and biomechanical properties in the seagrass *Zostera noltii*. Limnol. Oceanogr. 57, 1664–1672.
Lamb, J.B., van de Water, J.A., Bourne, D.G., Altier, C., Hein, M.Y., Fiorenza, E.A., Abu, N., Jompa, J., Harvell, C.D., 2017. Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. Science 355, 731–733.
Large, A., 2009. Aquatic ecosystems: trends and global prospects, edited by Nicholas V.C. Polunin. 2008. Cambridge University press: Cambridge, 482 p. ISBN: 978-0-521-83327-1. River Res. Appl. 25 (497–497). https://doi.org/10.1002/rra.1222.
Lee, K.S., Park, S.R., Kim, Y.K., 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. J. Exp. Mar. Biol. Ecol. 350, 144–175.
Lewis, D., Wallace, E., 1998. Program Developed for CO_2 System Calculations. Brookhaven National Laboratory, Upton, New York.
Martínez-Crego, B., Olivé, I., Santos, R., 2014. CO_2 and nutrient-driven changes across multiple levels of organization in *Zostera noltii* ecosystems. Biogeosciences 11, 7237–7249.
Masini, R.J., Manning, C.R., 1997. The photosynthetic responses to irradiance and temperature of four meadow-forming seagrasses. Aquat. Bot. 58, 21–36.
Massa, S.I., Arnaud-Haond, S., Pearson, G.A., Serrão, E.A., 2009. Temperature tolerance and survival of intertidal populations of the seagrass *Zostera noltii* (Hornemann) in southern Europe (Ria Formosa, Portugal). Hydrobiologia 619, 195–201.
Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowitz, R.M., 1973. Measurement of the aparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.
Moreno-Marín, F., Vergara, J.J., Pérez-Llorens, J.L., Pedersen, M.F., Brun, F.G., 2016. Interaction between ammonium toxicity and green tide development over seagrass meadows: a laboratory study. PLoS One 11, e0152971. http://dx.doi.org/10.1371/journal.pone.0152971.
Moreno-Marin, F., Brun, F.G., Pedersen, M.F., 2018. Additive response to multiple environmental stressors in the seagrass *Zostera marina* L. Limnol. Oceanogr. http://dx.doi.org/10.1002/lno.10789.
Netten, J.J.C., van der Heide, T., Smolders, A.J.P., 2013. Interactive effects of pH, temperature and light during ammonia toxicity events in *Elodea canadensis*. Chem. Ecol. 29, 448–458. http://dx.doi.org/10.1080/02757540.2013.769971.
Nicholls, R.J., Wong, P.P., Burket, V.R., Codignotto, J., Hay, J.E., McLean, R.F., Ragoonaden, S., Woodroffe, C.D., 2007. Coastal systems and low-lying areas. In: Clim. Chang. 2007 Impacts, Adapt. Vulnerability, pp. 315–356. http://dx.doi.org/10.1017/CBO9781107415379.
Nydahl, A., Panigrahi, S., Wikner, J., 2013. Increased microbial activity in a warmer and wetter climate enhances the risk of coastal hipoxia. FEMS Microbiol. Ecol. 85, 338–347. http://dx.doi.org/10.1111/1574-6941.12123.
Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.a., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996.
Ow, Y.X., Uthicke, S., Collier, C.J., 2016. Light levels affect carbon utilisation in tropical seagrass under ocean acidification. PLoS One 11, e0150352.
Palacios, S., Zimmerman, R., 2007. Response of eelgrass *Zostera marina* to CO_2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats. Mar. Ecol. Prog. Ser. 344, 1–13. http://dx.doi.org/10.3354/meps07084.
Pérez, M., Romero, J., 1992. Photosynthetic response to light and temperature of the seagrass *Cymodocea nodosa* and the prediction of its seasonality. Aquat. Bot. 43, 51–62. http://dx.doi.org/10.1016/0304-3770(92)90013-9.
Pérez, M., Romero, J., Duarte, C., Sand-Jensen, K., 1991. Phosphorus limitation of *Cymodocea nodosa* growth. Mar. Biol. 109, 129–133.
Pérez, F.F., Rios, A.F., Rellan, T., Alvarez, M., 2000. Improvements in a fast potentiometric seawater alkalinity determination. Cienc. Mar. 26, 463–478.
Prinn, R., Paltsev, S., Sokolov, A., Sarofim, M., Reilly, J., Jacoby, H., 2011. Scenarios with MIT integrated global systems model: significant global warming regardless of different approaches. Clim. Chang. 104, 515–537. http://dx.doi.org/10.1007/s10584-009-9792-y.
Quark, M.S.Y., Ziegler, A.D., Benner, S.G., Evans, S., Todd, P.A., Gillis, L.G., Vongtanaboon, S., Jachowski, N., Bouma, T.J., 2016. Processes affecting the spatial distribution of seagrass meadow sedimentary material on Yao Yai Island, Thailand. Estuar. Coast. Shelf Sci. 182, 136–145.
R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
Ralph, P.J., Durako, M.J., Enríquez, S., Collier, C.J., Doblin, M.A., 2007. Impact of light limitation on seagrasses. J. Exp. Mar. Biol. Ecol. 350, 176–193.
Rasheeh, M.A., McKenna, S.A., Carter, A.B., Coles, R.G., 2014. Contrasting recovery of shallow and deep water seagrass communities following climate associated losses in tropical north Queensland, Australia. Mar. Pollut. Bull. 83, 491–499.
Repolho, T., Duarte, B., Dionísio, G., Paula, J.R., Lopes, A.R., Rosa, I.C., Grilo, T.F., Caçador, I., Calado, R., Rosa, R., 2017. Seagrass ecophysiological performance under ocean warming and acidification. Sci. Rep. 7. http://dx.doi.org/10.1038/srep41443.
Ruiz, J.M., Marín-Guiraro, L., García-Muñoz, R., Ramos-Segura, A., Bernardeau-Esteller, J., Pérez, M., Sanmartí, N., Ontoria, Y., Romero, J., Arthur, R., Alcoverro, T., Procaccini, G., 2017. Experimental evidence of warming-induced flowering in the Mediterranean *Posidonia oceanica*. Mar. Pollut. Bull. http://dx.doi.org/10.1016/j.marpolbul.2017.10.037.
Ruiz-Frau, A., Gelcich, S., Hendriks, I.E., Duarte, C.M., Marbá, N., 2017. Current state of seagrass ecosystem services: research and policy integration. Ocean Coast. Manag. 149, 107–115.
Russell, B.D., Connell, S.D., Uthicke, S., Muehllehner, N., Fabricius, K.E., Hall-Spencer, J.M., 2013. Future seagrass beds: can increased productivity lead to increased carbon storage? Mar. Pollut. Bull. 73, 463–469. http://dx.doi.org/10.1016/j.marpolbul.2013.01.031.
Salo, T., Pedersen, M.F., 2014. Synergistic effects of altered salinity and temperatura on estuarine eelgrass (*Zostera marina*) seedling and clonals shoots. J. Exp. Mar. Biol. Ecol. 457, 143–150.
Sarmento, H., Montoya, J.M., Vázquez-Domínguez, E., Vaqué, D., Gasol, J.M., 2010. Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 12, 2137–2149. http://dx.doi.org/10.1098/rstb.2010.0045.
Schwarz, A.M., Björk, M., Buluda, T., Mtolera, M., Beer, S., 2000. Photosynthetic utilisation of carbon and light by two tropical seagrass species as measured in situ. Mar. Biol. 137, 755–761. http://dx.doi.org/10.1007/s002270000433.
Short, F.T., 1987. Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. Aquat. Bot. 27, 41–57. http://dx.doi.org/10.1016/0304-3770(87)90085-4.
Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. Aquat. Bot. 63, 169–196. http://dx.doi.org/10.1016/S0304-3770(98)00117-X.
Short, F.T., Polidoro, B., Livingstone, S.R., Carpenter, K.E., Bandeira, S., Bujang, J.S., Calumpong, H.P., Carruthers, T.J.B., Coles, R.G., Dennison, W.C., Erftemeijer, P.L.A., Fortes, M.D., Freeman, A.S., Jagtap, T.G., Kamal, A.H.M., Kendrick, G.A., Judson Kenworthy, W., La Nafie, Y.A., Nasution, I.M., Orth, R.J., Prathep, A., Sanciangco, J.C., van Tussenbroek, B., Vergara, S.G., Waycott, M., Zieman, J.C., 2011. Extinction risk assessment of the world's seagrass species. Biol. Conserv. 144, 1961–1971. http://dx.doi.org/10.1016/j.biocon.2011.04.010.
Silva, J., Barrote, I., Costa, M.M., Albano, S., Santos, R., 2013. Physiological responses of *Zostera marina* and *Cymodocea nodosa* to light-limitation stress. PLoS One 8 (11), e81058.
Soisson, L.M., Haanstra, E.P., van Katwijk, M.M., Asmus, R., Auby, I., Barillé, L., Brun, F.G., Cardoso, P.G., Desroy, N., Fournier, J., Ganthy, F., Garmendia, J.M., Godet, L., Grilo, T.F., Kadel, P., Ondiviela, B., Peralta, G., Puente, A., Recio, M., Rigouin, L., Valle, M., Herman, P.M.J., Bouma, T.J., 2018. Latitudinal patterns in European seagrass carbon reserves: influence of seasonal fluctuations versus short-term stress and disturbance events. Front. Plant Sci. http://dx.doi.org/10.3389/fpls.2018.00088.
Stitt, M., Krapp, A., 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ. 22, 583–621. http://dx.doi.org/10.1046/j.1365-3040.1999.00386.x.
Sullivan, B., Trevahan-Tackett, S., Neuhauser, S., Govers, L., 2017. Review: host-pathogen dynamics of seagrass diseases under future global change. Mar. Pollut. Bull. http://dx.doi.org/10.1016/j.marpolbul.2017.09.030.
Takahashi, M., Noonan, S.H.C., Fabricius, K.E., Collier, C.J., 2016. The effects of long term *in-situ* CO_2 enrichment on tropical seagrass communities at volcanic vents. ICES J. Mar. Sci. 73, 876–886.
Terrados, J., Ros, J.D., 1995. Temperature effects on photosynthesis and depth distribution of the seagrass *Cymodocea nodosa* (Ucria) Ascherson in a Mediterranean coastal lagoon: the Mar Menor (SE Spain). Mar. Ecol. 43, 133–144. http://dx.doi.org/10.1111/j.1439-0485.1995.tb00400.x.
Terrados, J., Duarte, C.M., Kamp-Nielsen, L., Agawin, N.S.R., Gacia, E., Lacap, D., Fortes, M.D., Borum, J., Lubanski, M., Greve, T., 1999. Are seagrass growth and survival constrained by the reducing conditions of the sediment? Aquat. Bot. 65, 175–197. http://dx.doi.org/10.1016/S0304-3770(99)00039-X.
Tomas, F., Martínez-Crego, B., Hernán, G., Santos, R., 2015. Responses of seagrass to anthropogenic and natural disturbance do not equally translate to irs consumers. Glob. Chang. Biol. 21, 4021–4030.
Udy, J.W., Dennison, W.C., Lee Long, W.J., McKenzie, L.J., 1999. Responses of seagrass to nutrients in the Great Barrier Reef, Australia. Mar. Ecol. Prog. Ser. 185, 257–271.
Unsworth, R.K.F., van Keulen, M., Coles, R.G., 2014. Seagrass meadows in a globally changing environment. Mar. Pollut. Bull. 83, 383–386. http://dx.doi.org/10.1016/j.marpolbul.2014.02.026.
Villazán, B., Pedersen, M.F., Brun, F.G., Vergara, J.J., 2013a. Elevated ammonium

concentrations and low light form a dangerous synergy for eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 493, 141–154.
Villazán, B., Brun, F.G., Jiménez-Ramos, R., Pérez-Lloréns, J.L., Vergara, J.J., 2013b. Interaction between ammonium and phosphate uptake rates in the seagrass *Zostera noltii*. Mar. Ecol. Prog. Ser. 488, 133–143.
Villazán, B., Salo, T., Brun, F.G., Vergara, J.J., Pedersen, M.F., 2015. High ammonium availability amplifies the adverse effect of low salinity on eelgrass *Zostera marina*. Mar. Ecol. Prog. Ser. 536, 149–162. http://dx.doi.org/10.3354/meps11435.
Villazán, B., Brun, F.G., González-Ortiz, V., Moreno-Marín, F., Bouma, T.J., Vergara, J.J., 2016. Flow velocity and light level drive non-linear response of seagrass *Zostera noltei* to ammonium enrichment. Mar. Ecol. Prog. Ser. 545, 109–121. http://dx.doi.org/10.3354/meps11631.
Waycott, M., Longstaff, B.J., Meleros, J., 2005. Seagrass population dynamics and water quality in the Great Barrier Reef region: a review and future research directions. Mar. Pollut. Bull. 51, 1–4.
Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. U. S. A. 106, 12377–12381. http://dx.doi.org/10.1073/pnas.0905620106.
Zeebe, R.E., Wolf-Gladrow, D.A., 2001. CO_2 in Seawater: Equilibrium, Kinetics, Isotopes (346 p). Elsevier, Amsterdam.
Zimmerman, R.C., Smith, R.D., Alberte, R.S., 1989. Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). J. Exp. Mar. Biol. Ecol. 130, 93–109. http://dx.doi.org/10.1016/0022-0981(89)90197-4.
Zimmerman, R.C., Kohrs, D.G., Steller, D.L., Alberte, R.S., 1997. Impacts of CO_2 enrichment on productivity and light requirements of eelgrass. Plant Physiol. 115, 599–607.